a concern is that T cells bearing immunogenic transgenes, such as those coding for mouse proteins, bacterial selection genes, or viral suicide genes, would be targeted by the recipient's immune system and deleted. We report now that we have reduced the risk of clearance of adoptively transferred genetically modified T cells due to a host-*versus*-graft immune-mediated reaction directed to the xenogenic components of the CAR and its vector. To decrease immunogenicity, we generated a CD19-specific CAR encoded by all-human transgenes (designated hCAR), wherein the murine CD19-specific scFv is replaced with a human scFv of a CD19-specific mAb, derived from mice immunized with CD19 and expressing human immunoglobulin genes. Primary human T cells were electro-transferred with the hCAR transgene, using *Sleeping Beauty* (SB) transposition, and co-cultured with artificial antigen presenting cells expressing CD19 antigen and co-stimulatory molecules, resulting in expansion and stable expression of hCAR without the need for concomitant drug selection. The $hCAR^+$ T cells could be detected by flow cytometry and Western blot analysis and demonstrated specific lysis of $CD19^+$ tumor targets. This report demonstrates technology for generating CD19-specific $hCAR^+$ T cells while avoiding expression of immunogenic proteins (e.g. murine scFv) and will likely be of interest to the many investigators considering CAR^+ T cells for treatment of tumors.

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COMPREHENSIVE TYPING OF 1143 SINGLE NUCLEOTIDE POLYMORPHISMS (SNP) IN 220 IMMUNOREGULATORY GENES DEMONSTRATES THAT POLYMORPHISMS IN CCL3, CCL4 AND CCL27 MODULATE THE RISK OF ACUTE GRAFT VERSUS HOST DISEASE (GVHD)

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A wide range of genetic polymorphisms has been studied in the context of hematopoietic stem cell transplantation. To date, most of these analyses have assessed a limited number of arbitrarily chosen SNPs in a single or limited number of genes. We created an Affymetrix custom SNP array and evaluated a total of 1143 SNPs in 220 distinct immune effector genes in 187 hematopoietic stem cell transplant (HSCT) recipients and their sibling donors. Where possible, SNPs that have been previously evaluated in the context of GVHD (e.g. IL-10 SNPs) were included on the array. Genomic DNA was extracted from peripheral blood mononuclear cells (PBMC) obtained from patients and their sibling donors. All patients were in remission at the time of sampling, all had undergone HSCT at the Dana-Farber Cancer Institute between 1998 and 2005 and all samples were drawn prior to transplantation. The transplants included myeloablative and non-myeloablative conditioning regimens, T cell depleted (TCD) and non-TCD grafts and sex matched and sex mis-matched donors. Using dChip software, we evaluated each of the SNPs on the array in patients and in their donors for an association between genotype and the development of acute GVHD. Univariate and multivariate statistical analyses were performed using the Cochran-Mantel-Haenzel test and a logistic regression model adjusting for age, sex mismatch and other transplant characteristics. The SNPs rs1063340 and rs1634508, that are believed to regulate the function of CCL3 and CCL4 respectively, were most highly associated with protection against aGVHD ($p < 0.003$), demonstrating an odds ratio (OR) of 0.6 for risk of acute GVHD in the logistic regression analysis. The SNP rs11575584, which is thought to regulate CCL27 was most highly associated with risk of aGVHD ($p = 0.005$), with an OR of 2 in the same logistic regression model. CCL3 and CCL4 are chemokine ligands for CCR5 while CCL27 is a skin-associated chemokine, which interacts with the CCR10 receptor. The CCR5 deletion mutation (which protects against HIV infection) has recently been associated with a reduced risk of aGVHD and increased epidermal expression of CCL27 has been shown in patients with cutaneous aGVHD. This study is the most comprehensive evaluation to date of functional genetic polymorphisms on the risk of aGVHD. It suggests that the CCL3-CCL4-CCR5 and CCL27-CCR10 pathways play important roles in the pathogenesis of aGVHD and further investigation of these pathways is warranted.

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ROLE OF STAT3 SIGNALING IN GVHD AND GVL

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Growing evidence suggests that STAT3 signaling in $CD4^+$ T-cells plays a key role in the pathogenesis of autoimmunity. We tested the role of the same pathway in the induction of GVHD and GVL response in a murine model of alloSCT. Using the MHC-matched B10.D2→BALB/c model in which GVHD is $CD4^+$ T-cell-mediated and has clinicopathologic features consistent with human chronic sclerodermatous GVHD, we examined the role of STAT3 signaling in $CD4^+$ T-cells in the pathogenesis of GVHD and GVL response. After conditioning (775 cGy) recipient mice received B10.D2 T-cell depleted (TCD) bone marrow (BM) and equivalent of 12×10^6 splenocytes (9.3×10^6 TCD splenocytes, repleted with 10^6 wild-type (WT) $CD8^+$ and 1.8×10^6 WT, or CD4-Cre × $STAT3^{flox/flox}$ ($STAT3^{KO}CD4^+$) T-cells), a dose shown to induce all signs of GVHD. We reproducibly induced all signs of chronic GVHD in chimeras receiving WT $CD4^+$ T-cells, but not in chimeras injected with $STAT3^{KO}CD4^+$ T-cells (median score of 0.0 *vs.* 5.2; $P < .001$). *In situ* studies showed that cutaneous GVHD was accompanied by prominent dermal infiltration of donor-derived inflammatory monocytes and complete turnover to donor $CD11c^+$ epidermal DC chimerism in chimeras receiving WT but not $STAT3^{KO}CD4^+$ T-cells ($P < .001$). Splenic $CD11c^+$ DCs, $CD4^+$ and $CD8^+$ T-cell chimerism was nearly completely donor-derived and did not differ between the two sets of described chimeras. We also found that in this model, pathogenic $CD4^+$ T-cells do not acquire T_H17 phenotype and that STAT3 signaling disruption leads to expansion of $Foxp3^+CD4^+$ T-cells. To examine the role of STAT3 pathway in eliciting GVL response, we developed a model of preestablished disease in which 10^6 A20 lymphoma cells were administered to recipients 10 days prior to alloSCT. Addition of GVH inoculum containing $STAT3^{KO}CD4^+$ T-cells enabled potent GVL response when compared to animals receiving only TCD BM, or BM with added TCD SPL ($P < .001$). However, it was inferior to that observed in the animals receiving WT $CD4^+$ T-cells ($P < .001$). Our findings indicate that: a) intact STAT3 signaling in $CD4^+$ T-cells is required for clinical manifestations of GVHD in B10.D2→BALB/c model; b) commitment of $STAT3^{KO}CD4^+$ T-cells to regulatory $Foxp3^+$ lineage is likely a result of their insensitivity to IL-6 in the abundance of TGF-β; c) STAT3 ablation enables preservation of GVL, while reducing clinical manifestations of GVHD. Further exploration of the role of STAT3 pathway in posttransplant events is required.

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SIMILAR GRAFT-VERSUS-LEUKEMIA EFFECT USING MATCHED UNRELATED DONORS, COMPARED TO HLA-IDENTICAL SIBLINGS FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION

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